Official journal website:
amphibian-reptile-conservation.org

Amphibian & Reptile Conservation
15(2) [General Section]: 156–171 (e286).

Advertisement calls of six glassfrog species in the Colombian Andes, and comments on priorities for future research and conservation

[1,2,3,*]Angela M. Mendoza-Henao, [4]Sebastián Duarte-Marín, and [5]Marco Rada

[1]Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-153, 04510 Mexico City, MEXICO [2]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Apartado Postal 70-153, C.P. 04510, Mexico City, MEXICO [3]Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Claustro de San Agustín - Carrera 8 #15-08, Villa de Leyva, Boyacá, COLOMBIA [4]Grupo Evolución, Ecología y Conservación (EECO), Programa de Biología, Universidad del Quindío, Armenia, COLOMBIA [5]Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, BRAZIL

***Abstract.*—Conservation initiatives are particularly important in light of the high proportion of species threatened with extinction worldwide. Advertisement calls are a valuable resource for conservation given their importance for studies on amphibian taxonomy, systematics, evolutionary biology, ecology, and monitoring strategies. However, advertisement calls are still unknown for many species. Here, advertisement call descriptions are provided for six glassfrog species (*Centrolene huilensis*, *Centrolene hybrida*, *Nymphargus orenonympha*, *Rulyrana flavopunctata*, *Rulyrana susatamai,* and *Sachatamia punctulata*) from six localities in four municipalities in the Andes of Colombia. Based on our current knowledge of the acoustics, distribution ranges, presence in protected areas, and IUCN Red List threat status, some species of glassfrogs are identified as priorities for future studies. The overall parameters measured for the advertisement calls of the species described here fall within those known for other species from the same genus, but the species studied here are differentiated from their congenerics by the pulse rate, number of notes, and/or dominant frequency. To date, advertisement calls are known for approximately 60% of glassfrog species and we identified 23 priority species with unknown calls which should be the focus of future research efforts.**

Keywords. Anura, bioacoustics, Centrolenidae, South America, threatened species, taxonomy

***Resumen.*—Las iniciativas de conservación son especialmente importantes teniendo en cuenta la elevada proporción de especies amenazadas de extinción en todo el mundo. Dada su importancia para los estudios sobre taxonomía, sistemática, biología evolutiva, ecología y estrategias de seguimiento de los anfibios, los cantos de anuncio son un recurso valioso para la conservación. Sin embargo, los cantos de anuncio siguen siendo desconocidos para muchas especies. En este trabajo se describen los cantos de anuncio de seis especies de ranas de cristal (*Centrolene huilensis, Centrolene hybrida, Nymphargus orenonympha, Rulyrana flavopunctata, Rulyrana susatamai* y *Sachatamia punctulata*) de seis localidades en cuatro municipios de los Andes de Colombia. A partir de los conocimientos sobre acústica, áreas de distribución, presencia en áreas protegidas y estado de amenaza (Listas Rojas de la UICN), se identificaron algunas especies de ranas de cristal como prioritarias para futuros estudios. Los parámetros generales medidos en los cantos de anuncio de las especies descritos están dentro de los conocidos para otras especies del mismo género, pero las especies se diferencian de sus congéneres por la frecuencia de los pulsos, el número de notas o la frecuencia dominante. Hasta la fecha, se conocen los cantos de anuncio de aproximadamente el 60% de las especies de ranas de cristal y hemos identificado 23 especies prioritarias con llamadas desconocidas hacia las que sugerimos dirigir los esfuerzos de investigación.**

Palabras clave. América del Sur, Anura, bioacústica, Centrolenidae, especies amenazadas, taxonomía

Citation: Mendoza-Henao AM, Duarte-Marín S, Rada M. 2021. Advertisement calls of six glassfrog species in the Colombian Andes, and comments on priorities for future research and conservation. *Amphibian & Reptile Conservation* 15(2) [General Section]: 156–171 (e286).

Accepted: 2 May 2021; **Published:** 12 October 2021

Correspondence. **am.mendozah@gmail.com*

Introduction

Amphibians provide valuable ecosystem services in aquatic and terrestrial environments acting in disease control, pest control, and nutrient cycling (Valencia-Aguilar et al. 2013), as well as providing an important element of terrestrial and aquatic food webs (Zipkin et al. 2020). For instance, the tadpoles of some glassfrog species are considered important for nutrient cycling in aquatic ecosystems by stimulating the fungal activity in the leaf litter (Connelly et al. 2011). Therefore, the absence of these species can alter the structures and functions of the ecosystems (Whiles et al. 2006, 2013). At the same time, amphibians are the most threatened group of vertebrates worldwide, being affected by habitat destruction, climate change, and epidemic diseases (Lips et al. 2005; Loyola et al. 2012; Pounds et al. 2006; Wake and Vredenburg 2008). Habitat fragmentation threatens 89% of Neotropical amphibians through population isolation, inbreeding, and edge effects (Stuart et al. 2008), and the proportions of declined species which exhibit recovery, stabilize at lower abundance, or continue to decline remain unknown (Scheele et al. 2019).

Considering that economic resources directed towards conservation are scarce, these efforts must be channeled towards the most vulnerable species (Barret et al. 2014). Multiple approaches have been employed to identify conservation priorities, including selection by species range, phylogenetic endemism, or coverage by protected areas (Mendoza and Arita 2014). Protected areas mitigate threats acting upon anuran populations, mainly by decreasing the deforestation due to land-use changes. Additionally, protected areas also facilitate scientific research by allowing investigators to perform long-term studies on the biology and ecology of the key species (Brooks et al. 2004; Guerra et al. 2018; Guayasamin et al. 2020; Lips et al. 2005; Loyola et al. 2012).

Detailed descriptions of advertisement calls are a valuable resource among the multidisciplinary approaches for clarifying taxonomic limits (e.g., Padial and De La Riva 2009; Köhler et al. 2017), and for studies of behavior, reproductive ecology, and evolution (Wells 2007). Advertisement calls are also an important resource for the effective conservation assessment, planning, and management of threatened species (Laiolo 2010; Sanchez-Giraldo et al. 2020). However, despite increasing efforts in the description of frog calls in neotropical species-rich countries (Hutter et al. 2014; Guayasamin et al. 2020; Viuche-Lozano et al 2018), information on the advertisement calls is still lacking for many species (Guerra et al. 2018; Rivera-Correa et al. 2021).

The glassfrogs (family Centrolenidae) are a significant component of the neotropical amphibian communities in rivers and streams. Glassfrogs comprise a highly diverse group, currently with 12 genera and 156 species (Guayasamin et al. 2020; Frost 2021). The northern Andes in Colombia, Ecuador, Peru, and Venezuela have very high species richness and endemism rates (Guayasamin et al. 2020; Ruiz-Carranza and Lynch 1991a; Twomey et al. 2014). Glassfrogs include riparian frogs found from sea level in tropical rainforests to 3,300 m asl in the Andean Páramos, where most species call to attract females, to display territoriality, and as part of parental care behavior (Cisneros-Heredia and McDiarmid 2007; Delia et al. 2014; Bravo-Valencia and Delia 2016; Delia et al. 2017). Nevertheless, the vocalizations of most glassfrogs remain largely unknown and are limited to approximately 92 of the 156 species (or about 59%).

The large diversity in this region is threatened by human activities such as mining, exploitation of natural resources, and land transformation, all of which have significant consequences on water quality and, therefore, on the associated diversity. More than one-third of the 156 glassfrog species are in one of the risk categories of the International Union for Conservation of Nature, including eight Critically Endangered, 21 Endangered, and 23 Vulnerable, with another 53 in the Data Deficient category (IUCN 2010). Unfortunately, the areas with higher diversity and endemism are not effectively covered within established protected areas (Mendoza and Arita 2014).

Considering the need for correctly identify glassfrog species, especially distinguishing those that are sympatric or morphologically similar, here the advertisement calls of four glassfrog species in three genera from the Colombian Andes are described for the first time, and call information for two *Rulyrana* species from additional localities are provided. Finally, considering that the description of the advertisement calls is necessary for conducting field research through active and passive searches, and that research and conservation efforts must be channeled towards the most vulnerable species, we also provide a comprehensive evaluation of the current status of glassfrog call descriptions regarding the species conservation status to identify the priority species for future studies.

Materials and Methods

Collection Sites

Nocturnal fieldwork occurred in April and May 2018 in the Andes of Colombia (i.e., Central and Eastern cordilleras; Fig. 1). Advertisement calls of six species were recorded from six localities in four municipalities: (1) *Centrolene hybrida*: Miraflores municipality in Boyacá department, at 1 km N of the finca el Vergel, 38 km, by road to ENE Garagoa (5.10089, -73.22038; 1,947 m; 27–29 April 2018); (2) *Centrolene huilensis*: Isnos municipality in Huila department at 5 km at NW of Istos in the road to Popayán (1.96838, -76.25021; 1,988 m; 4–5 May 2018); (3) *Nymphargus oreonympha*: Alto Gabinete, La Ruidosa stream, in Florencia municipality in Caquetá department, road Florencia-Altamira (1.86936, -75.67183; 2,073 m, 6–7 May 2018); (4) *Rulyrana flavopunctata*: Sucre

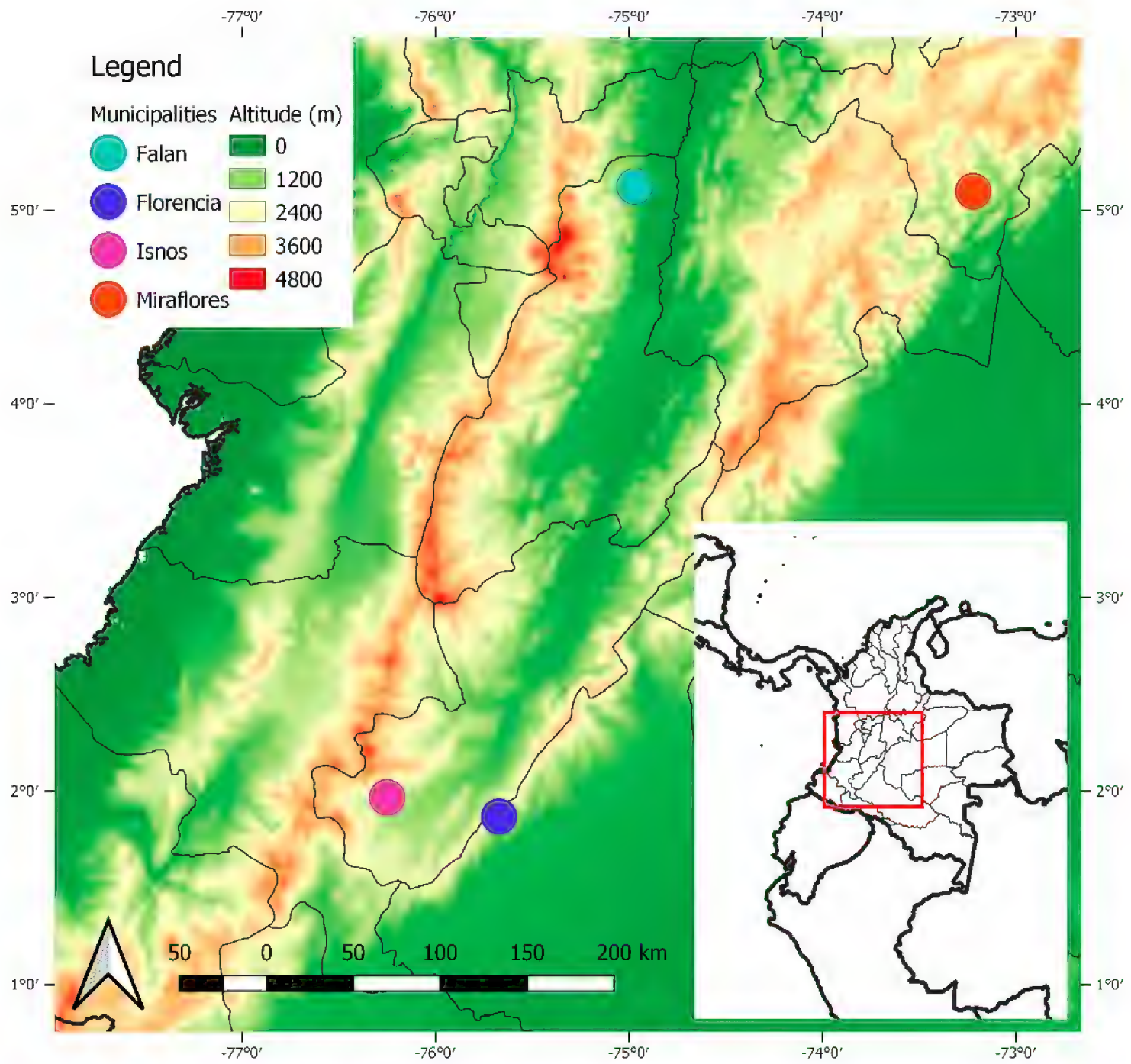


Fig. 1. Fieldwork localities in the Central and Eastern Cordilleras where the advertisement calls were obtained.

sidewalk, Florencia municipality in Caqueta department, road Florencia-Altamira (1.86936, -75.67183; 1,134 m; 8 May 2018); (5) *Rulyrana susatamai*: Falan municipality, Murillo stream in Piedecuesta, Tolima department (5.1258, -74.97052; 1,133 m; 23–25 April 2018); and (6) *Sachatamia punctulata*: Falan municipality, Cuamo River at 10 km southwards from Falan head, in Tolima department (5.1258, -74.97052; 441 m; 23–25 April 2018).

At each field site, free surveys (not time or effort restricted) were conducted along the rivers and streams. At least ten calls per individual were recorded in WAV format with a digital recorder (Tascam DR-40) and a unidirectional microphone (Sennheisser K6/ME 66) at a sampling rate of 44.1 kHz and an amplitude resolution of 16 bits. Species identities were verified by comparison of morphological characters with the species diagnoses provided in Ruiz-Carranza and Lynch (1991 b,c; 1995a,b). Microhabitat details were recorded, such as calling perch height, perch type, and substrate. Surface temperatures of either male frogs or the substrate from which a male frog recently jumped were recorded using an infrared digital thermometer (Benetech GM300, resolution 0.1 °C). Snout-vent length (SVL) of calling males was measured with a digital caliper. Voucher specimens were fixed with 10% formalin. Field numbers of Angela M. Mendoza-Henao (AMMH) are reported for those specimens already deposited in the Instituto de Ciencias Naturales (ICN), but not yet allocated museum numbers. Note that due to the COVID-19 pandemic, the ICN is shut down and numbers for AMMH specimens mentioned in the manuscript are currently not available. After allocation of museum numbers to these specimens, they can be requested from the corresponding author. Recordings used for call descriptions were deposited (in WAV format) in the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt (IAvH-CSA-34233 to 34250).

Calls were analyzed using the software Raven Pro 1.4 (http://ravensoundsoftware.com/) with Blackman window type, window size of 5 ms, 80% overlap, and DFT size of 1,024 samples to obtain the dominant frequency (in Hz). In cases of multiple notes per call, the inter-note silent interval was measured and for pulsed calls the pulse rate was determined. Temporal parameters were measured from the waveform (in ms). The sampling unit for the descriptive analysis was the individual. Call figures of the spectrograms were generated using Seewave v. 1.6 package (Sueur et al. 2008) in the R platform (version 3.4.3; R Development Core Team 2014). The call terminology of Köhler et al. (2017) was followed to categorize vocalization types, temporal variables, and spectral variables. In that sense, we followed the note-centered approach, and pulsed notes were defined as those notes composed by multiple short

(less than 50 ms) undividable sound units spaced with silent intervals within the note.

Status of Glassfrogs Call Descriptions

To compile the information available in published studies (peer-reviewed papers and books) for described advertisement calls, online databases (AmphibiaWeb, BioWeb Ecuador) were searched for call information, and the Google Scholar search engine was queried with the terms "advertisement call," "glassfrog," "glass frog," and "Centrolenidae." Included here are the species calls described in the sources identified, and those from an on-going study (Duarte-Marin et al., submitted). For each species, the information on level of threat was obtained based on their IUCN (2019) conservation status. Because many species are either classified as Data Deficient or simply Non-Evaluated, we complemented the IUCN information with two additional parameters in our comparisons: level of endemism and occurrence in protected areas.

For the level of endemism, the range per species was obtained and four categories were defined: Local, covering less than 1,500 km^2 of distribution at 1–5 locations in 1–2 countries; Sub-regional, area 1,501–35,000 km^2 in 1–3 countries without entirely covering a whole recognized geographical region (e.g., Chocó, Northern Andes); Regional, area 35,001–200 000 km^2, distributed in a large part of a country, in more than two countries, and distributed in a large part or totality of a geographical region; Global: covering more than 200,000 km^2, distributed in a whole region or several geographical regions. For occurrence in protected areas, the species ranges were compared with the polygons of the protected areas of all categories included in the World Database of Protected Areas, WDPA (IUCN/UNEP 2010). Using Spatial Analysis tools in the ArcGIS software (ESRI 2007), the proportion of each species range contained in a protected area was determined. These values were then grouped by the criteria of Rodrigues et al. (2004): a species with a range smaller than 1,000 km^2 should have 100% of its range within protected areas; and a species with a range larger than 250,000 km^2 should have at least 10% of its range within protected areas. For species with intermediate range sizes, the critical percentage was calculated by linear interpolation from these two extremes.

The results of the three criteria (threat category, endemism, and occurrence in protected areas) were combined for each species and priority species were defined as those with local ranges (i.e., less than five known locations in less than 1,500 km^2), not covered by protected areas, and in either the Critical, Endangered, Data Deficient or Non-Evaluated categories of IUCN.

Results

In total, 250 advertisement calls were analyzed for 19 individuals of six glassfrog species. Detailed information for the voucher code, temperature at the time of recording, number of individuals recorded, mean snout-vent length (SVL), air temperature, and measured call traits for each individual is provided in Table 1. Values in the following call descriptions are given as mean ± standard deviation. Spectrograms and oscillograms of the advertisement call

Table 1. Call features for the six glassfrog species described in this study. N = number of analyzed calls (recorded males). Values are mean ± SD. The call recordings from collected and non-collected specimens were deposited in the Colección de Sonidos Ambientales Mauricio Álvarez-Rebolledo.

	Centrolene huilensis	*Centrolene hybrida*	*Nymphargus oreonympha*	*Rulyrana flavopunctata*	*Rulyrana susatamai*	*Sachatamia punctulata*
N: calls (males)	58 (5)	40 (4)	60 (3)	18 (1)	34 (3)	40 (3)
Mean Snout-Vent Length (mm)	27.0	21.5	24.5	19.8	22.8	27.8
Collector field numbers	AMMH 174–178	AMMH 168–170	AMMH 181–183	AMMH 186	AMMH 162–164	AMMH 165–167
Call vouchers	IAvH-CSA 34233–34237	IAvH-CSA 34328–34340	IAvH-CSA 34241–34242	IAvH-CSA 34244	IAvH-CSA 34245–34247	IAvH-CSA 34248–34250
Temperature (°C)	14.7	12.5	12.6	17.0	19.5	23.5
Dominant frequency (Hz)	4,785.5 ± 336.4 (first note), 4,940.2 ± 266.3 (second note)	6,072.9 ± 106.0	4,085.0 ± 134.55	6,361.9 ± 35.6	5,967.2 ± 163.6	5,611.7 ± 213.9
Call duration (ms)	142.8 ± 4.41	227.8 ± 61.22	14.3 ± 2.2	29.7 ± 3.1	58.7 ± 6.58	183.2 ± 155.6
Number of notes	1 or 2 (1.9 ± 0.31)	1 to 5 (1.2 ± 0.68)	1	1	1	1 to 5 (2.35 ± 1.14)
Note duration (ms)	28.8 ± 1.06	19.9 ± 9.7	14.3 ± 2.2	29.7 ± 3.1	58.7 ± 6.58	17.6 ± 4.2
Internote interval (ms)	97.5 ± 12.8	1,322.3 ± 87.6	—	—	—	97.2 ± 16.1
Call type	Tonal	Tonal	Tonal	Pulsar	Pulsar	Pulsatic-harmonic
Pulses per note	—	—	—	4.06 ± 0.54	8.41 ± 0.82	3.74 ± 1.62

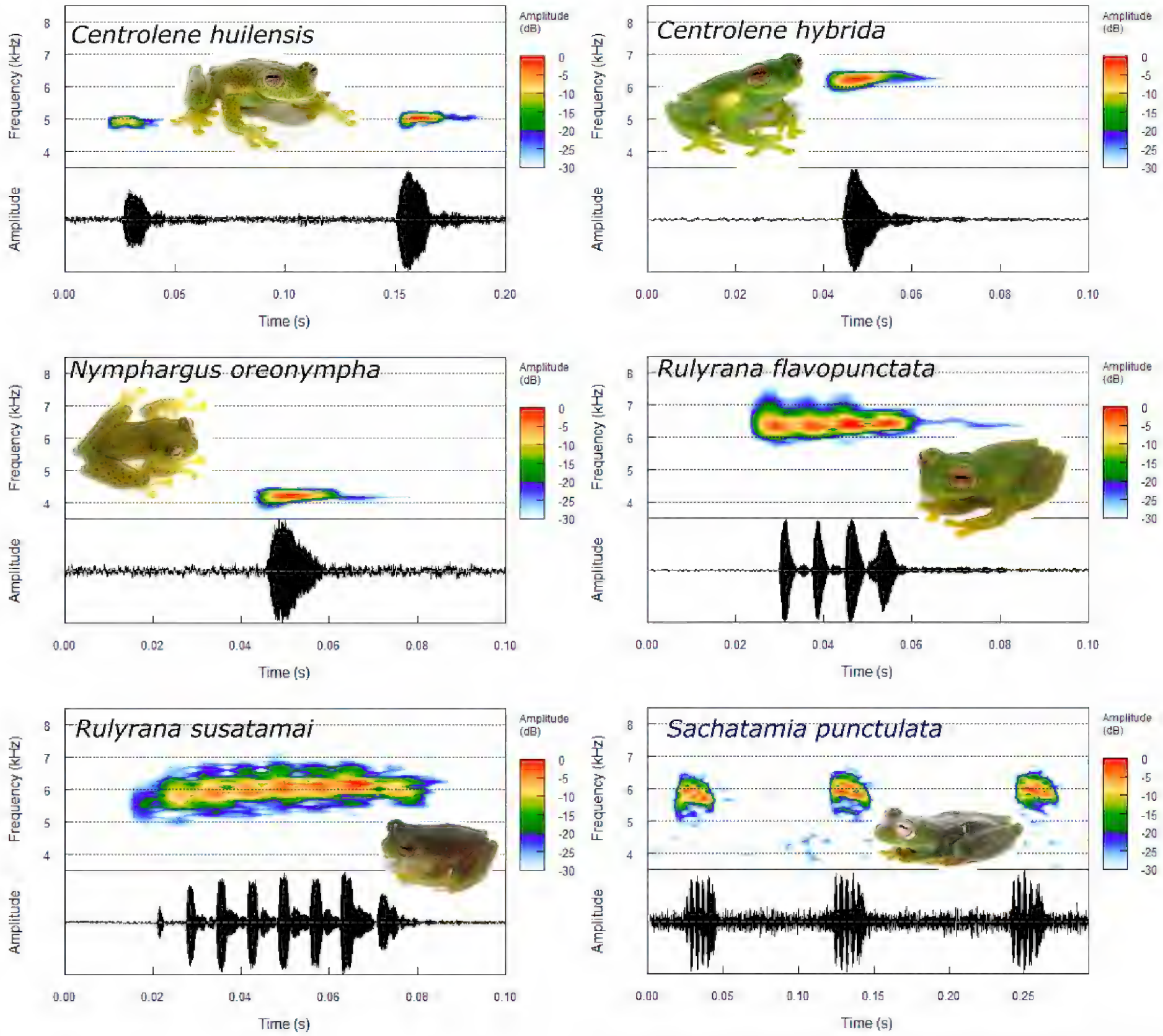


Fig. 2. Spectrograms (top) and oscillograms (bottom) of the described glassfrogs advertisement calls. All calls are displayed at Blackman window (length = 512) and 80% overlap, at the same frequency range, and all but *S. punctulata* and *C. huilensis* are displayed at the same temporal scale.

of each species are provided in Fig. 2.

Centrolene huilensis. Individuals were found calling from the upper side of leaves at 1.5 to 4 m above the water. Calls were obtained between 2300 h and 0130 h on clear nights with a full moon. The advertisement call is composed of one or two single-toned notes similar to a "Tic" to the human ear. The first note has a duration of 27.63 ± 11.37 ms and a peak frequency of 4,785.5 ± 336.42 Hz while the second one has a duration of 30.9 ± 11.85 ms and a peak frequency of 4,940.2 ± 266.34 Hz (Fig. 2). Both notes have limited amplitude modulation with the highest energy located near the middle of the note. The notes are separated by a silent interval of 9.75 ± 1.28 ms.

Centrolene hybrida. Individuals call from the upper surfaces of leaves from 20 cm to 5 m above the water. Advertisement calls were obtained between 2000 h and 2350 h on clear nights in vegetation above small and medium-sized streams located on the sides of cattle-cleared areas. The call is a toned note similar to a "Tic" to the human ear. The whole note lasted 19.9 ± 9.7 ms and had a dominant frequency of 6,072.9 ± 106.0 Hz (Fig. 2). In most cases, the call was composed by a single note, but in a few instances, individuals produced calls with up to five notes after a silent interval of 1,322.3 ± 87.6 ms. The highest energy is displayed at the first third of the note.

Nymphargus oreonympha. Individuals were recorded calling at heights of up to 4 m in leaves and branches above the water in a small stream between 2200 h and 0115 h, with some males near egg depositions. Calls were obtained on nights following a heavy afternoon rain. The call is a single-toned short note (14.3 ± 22.1 ms) similar to a "Tic" to the human ear. The dominant frequency was 3,995.9 ± 37.8 Hz, and low in comparison with the other calls described here. The notes have limited amplitude modulation with the highest energy located near the middle of the note. One individual showed a call with a

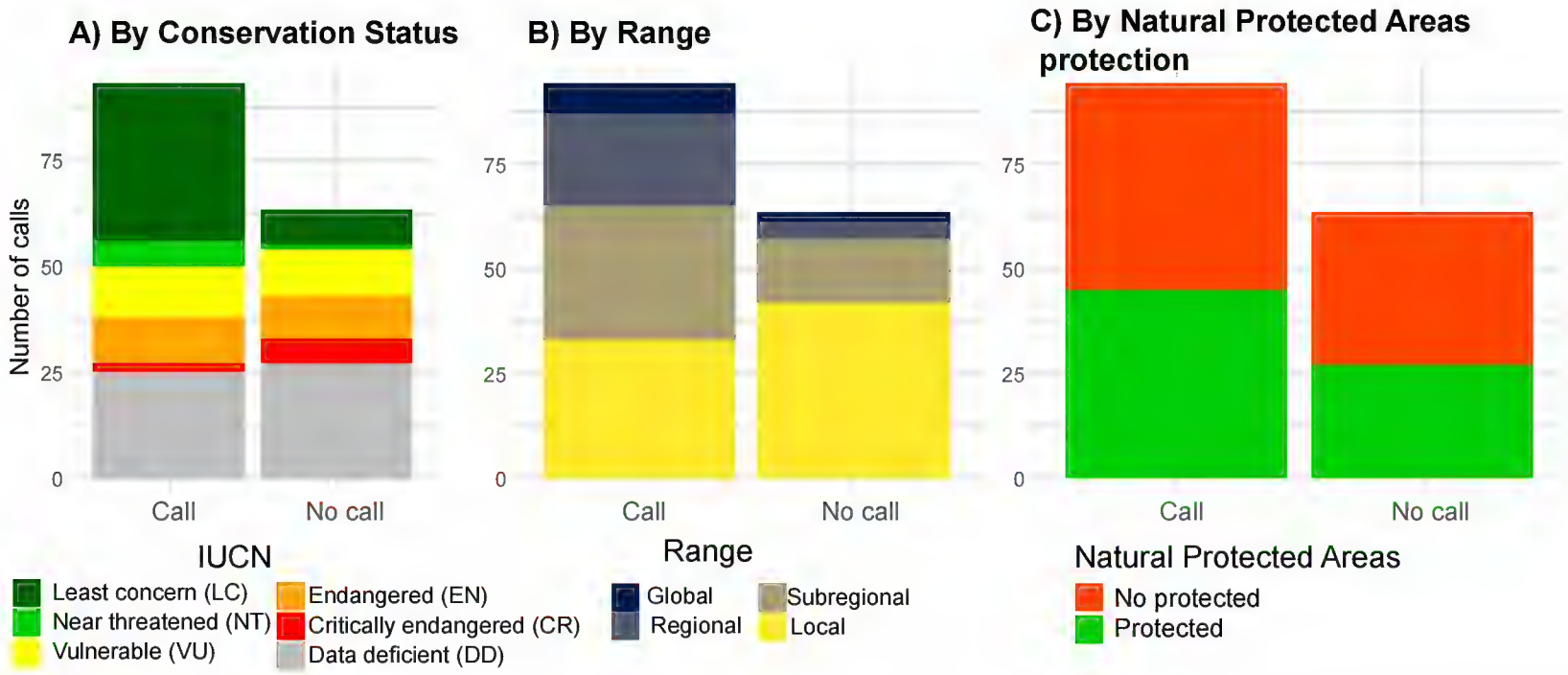


Fig. 3. Counts of glassfrog species for those with and without the call described, and categorized by: **(A)** IUCN conservation status categories; **(B)** Endemism or distribution range (see details in **Materials and Methods**); and **(C)** Occurrence in National Protected Areas.

slightly higher dominant frequency (4,263.3 ± 55.8 Hz) after a confrontation with another male. After the combat, the male emitted the call at 20 cm above the water on a branch and the second individual left the branch.

Rulyrana flavopunctata. The specimen calling was recorded at 0226 h from the upper side of a large Araceae-like leaf at 1 m above the water. Several males of the species were observed calling together with other individuals in the background. The call is a single-trilled note similar to a "Trii" to the human ear, lasting 29.7 ± 3.1 ms, with 3–5 pulses (mean 4.06) at a rate of 137.9 pulses/s, and the dominant frequency was 6,361.8 ± 35.6 Hz with no amplitude modulation (Fig. 2).

Rulyrana susatamai. The specimens were calling from 2000–0340 h during nights with light rain. Individuals of *R. susatamai* were calling from the tips of the upper sides of leaves, above small streams at heights of around 2–5 m. The call is a single-trilled note similar to a "Trii" to the human ear, lasting 58.7 ± 6.58 ms with a dominant frequency of 5,967.2 ± 163.6 Hz (Fig. 2). The note comprises 7–10 pulses at a rate of 143.3 pulses/s whose energy increases slightly along the note.

Sachatamia punctulata. The calls were emitted from rocks and on vegetation up to 4 m high between 0100 and 0300 h. After heavy rains, multiple amplectant individuals were also observed. The call is a series of pulsatic-harmonic notes (1–5 notes) of 17.6 ± 4.2 ms, with an internote interval of 97.2 ± 16.1 ms and a dominant frequency of 5,611.7 ± 213.9 Hz with no amplitude modulation detected (Fig. 2). It is similar to a "Trii" to the human ear. Each note shows up to 3.74 ± 1.62 pulses at a rate of 220 pulses/s.

Status of Glassfrog Call Descriptions

Including the advertisement calls of the species described here, the number of species with advertisement call descriptions increases to 92 (59.3% of all species of glassfrogs, see Appendix 1). Regarding the species by conservation status (Fig. 3A), 48 of the 55 of non-threatened species (Least Concern and Not Evaluated) have their calls described, while 30 of the 95 threatened species (Vulnerable, Endangered, Critically Endangered) have their calls described. Regarding the distribution categories (Fig. 3B), the call is known for 63 of the 82 species that have Sub-regional, Regional, and Global distributions; whereas only 37 of the 75 species with a Local distribution have their calls described. Fifty of the species with their calls described are effectively covered by Natural Protected Areas while the call is unknown for 35 species restricted to outside of Natural Protected Areas (Fig. 3C). By combining all these criteria, a total of 23 species whose call is undescribed are identified as priorities for bioacoustics and conservation research (Appendix 2).

Discussion

The detailed descriptions of the advertisement calls for the anurans of highly diverse regions, like the northern Andes, are a valuable resource for studies in taxonomy, biodiversity monitoring, and various ecological and evolutionary aspects of anurans. Here, the descriptions of the advertisement call of six Andean glassfrog species are provided, and the call parameters are compared with those from other species in the same genus. The species which either do or do not have their calls described are assessed regarding their conservation status and the implications and challenges of the current species coverage are discussed in terms of ecology and conservation.

Call Comparisons among Species

The overall structures of the advertisement calls of the species included in this study coincide with those known for other glassfrogs in terms of having short notes (less than 200 ms) of relatively high frequencies (over 3,000 Hz), but the specific calls can be differentiated among congeneric species. For instance, the advertisement call of *S.*

punctulata differs from the calls described for *Sachatamia ilex* and *Sachatamia albomaculata* by having a set of multiple notes (1–5), while those of the latter species are composed only by a single note (Guayasamin et al. 2020; Kubicki 2007). The call of *N. oreonympha* has a frequency of 4,085.0 ± 134.55 Hz, similar to most calls described for *Nymphargus* species (only *N. siren* has a frequency over 5,000 Hz, Guayasamin et al. 2020), however the *N. oreonympha* advertisement call can be differentiated from the others described to date (a) by having the shortest note (14.3 ± 2.2 ms vs. 202.3 ± 9.3 ms in *N. bejaranoi,* 115.0 ± 18.0 ms in *N. grandisonae,* 122.0 ± 9.0 ms in *N. griffithsi,* 26.0 ± 6.0 ms in *N. lasgralarias,* 16.5 ± 2.1 ms in *N. mariae,* 100.0 ± 7.0 ms in *N. manduriacu,* 181 ± 2.81 ms in *N. pluvialis,* 24.0 ± 7.0 ms in *N. siren,* and 170.1 ± 4.41 ms in *N. truebae*); and (b) because the *N. oreonympha* advertisement call is comprised by a single tonal note while most others are pulsar (*N. bejaranoi, N. gradisonae, N. manduriacu, N. pluvialis,* and *N. truebae*; Catenazzi et al. 2009; Guayasamin et al. 2019, 2020; Hutter and Guayasamin 2012; Márquez et al. 1996).

Centrolene, with 24 species described, is one of the high diversity clades within Centrolenidae; it is also one of the genera with more advertisement calls recorded. The advertisement calls of *Centrolene* species usually consist of pulsed notes (e.g., *C. altitudinale*, *C. buckleyi*, *C. condor*, *C. geckoidea*, *C. sabini*, *C. hesperia*, *C. notosticta*, *C. peristicta*, *C. sanchezi,* and *C. venezuelense*), a combination of pulsed and tonal notes (*C. lynchi*), or tonal and pulsed notes as in *"C." quindianum* (Cadle and McDiarmid 1990; Márquez et al. 1996; Grant et al. 1998; Señaris and Ayarzagüena 2005; Guayasamin et al. 2006; Dautel et al. 2011; Catenazii et al. 2009; Almendariz-C and Batalla 2012; Salgado and Guayasamin 2018; Viuche-Lozano et al. 2018; Rios-Soto et al. 2017). This call structure differs of that observed here in *C. huilensis* and *C. hybrida,* and those described for *C. daidalea* and *C. savagei*, whose calls consist of tonal note(s) (Cardozo-Urdaneta and Señaris 2012; Vargas-Salinas et al. 2017). Likewise, we can note that among *Centrolene* species, the advertisement calls of *C. huilensis*, *C. altitudinale, C. hybrida,* and *C. sanchezi* are quite similar in several spectral parameters (see Señaris and Ayarzagüena 2005; Guayasamin et al. 2020). However, there are remarkable differences in some temporal parameters of the calls, i.e., the internote interval of *C. huilensis* is shorter (97.5 ± 12.8 ms) than that reported in *C. altitudinale* (331 ± 6.5 ms) and the notes of *C. hybrida* are longer (227.8 ± 6122 ms) than those described for *C. sanchezi* (11 ± 2.8 ms). In addition, in most cases the advertisement calls of *C. hybrida* exhibited a single note (mean 1.2 notes per call), while in *C. sanchezi* the mean was 5.9 notes. Although most glassfrog calls comprise high-pitched short notes of high energy without variation between the dominant frequencies of each note, the call of *C. huilensis* had variation in the dominant frequencies between the two notes. To date, this pattern is known for only a few glassfrog species, such as *C. lynchi* (Dautel et al. 2011), *C. hesperium* (Cadle and McDiarmid 1990), and *C. sabini* (Catenazzi et al. 2009).

The delimitation of some species within *Rulyrana* is still a matter of debate. For example, Twomey et al. (2014) found a very low genetic distance between samples of the sister species *R. adiazeta* and *R. susatamai,* raising some questions regarding their taxonomic status. The species are highly similar morphologically, so a multidisciplinary approach is necessary to resolve the species limits, and an integrative approach is necessary to determine the phylogenetic relationships between *R. susatamai* and *R. adiazeta* (Twomey et al. 2014). In this sense, information for the species in their respective type localities can be used to solve such riddles. Here, the parameters of the advertisement call of *R. susatamai* obtained from the type locality (Falan municipality, Tolima department) are quite similar to those described by Galindo et al. (2020) from males of the species recorded in two localities also in Tolima department (Anzoátegui and Líbano municipalities). They will be useful for direct comparisons in order to resolve this taxonomic problem when the advertisement call of *R. adiazeta* becomes available. Concerning *R. flavopunctata*, although the male recorded here (eastern slope of eastern Cordillera in Colombia, AMMH-186, Fig. 2) is morphologically similar to males from Ecuador, the advertisement calls described by Guayasamin et al. (2020) have a longer mean note duration (40 ms Ecuador vs. 19.9 ms Colombia), greater mean number of pulses per note (7 Ecuador vs. 4 Colombia), and greater mean pulse rate (262 pulses/s Ecuador vs. 137.9 pulses/s Colombia). In this study, an unexpected variation in parameters is illustrated between two geographically distant advertisement calls. Temporal acoustic parameters, such as call duration and number of pulses, are highly influenced by climatic and social factors (Morais et al. 2012; Köhler et al. 2017). Unfortunately, the number of individuals recorded in both localities was too low to provide an explanation for these large differences, which could potentially be interpreted as individual or intraspecific variation or as evidence of undescribed morphologically similar diversity. However, the variation detected is a good example highlighting the need to include calls from different individuals at a variety of localities in taxonomical and ecological studies.

Usefulness and Gaps in Conservation Strategies

The advertisement calls described in this study represent a reliable resource for further studies on glassfrog species. Two species, *S. punctulata* and *R. susatamai*, are considered Vulnerable and Near Threatened by the IUCN, respectively. They were previously identified as priority species for conservation considering their high phylogenetic endemism (Twomey et al. 2014; Mendoza and Arita 2014). We also described for the first time the call of the Endangered species *C. huilensis*, which was recently registered for Colombia after 30 years without records. Populations of *C. huilensis*, especially those from

Ecuador, require further evaluation regarding taxonomy and ecological monitoring (Mendoza-Henao et al. 2019). The call description provided here will facilitate species identification and detection in acoustic surveys, as a valid source of data on the species distribution which is needed for conservation (Carvajal-Endara et al. 2019).

Although the number of studies of bioacoustics in glassfrogs has increased in recent years, there are still many species in this family for which the advertisement call is still undescribed. Six of the eight Critical Endangered species that do not have their advertisement call described are not recorded in any protected areas (Fig. 3). In addition, 23 species are under some degree of threat and thus considered priorities for conservation (i.e., assessed in an IUCN threat category, having a local distribution, and not being covered by a protected area, Appendix 1).

The current gaps in glassfrog call descriptions can be explained by several factors. Many locations where the species are reported are difficult to access. Considering that some glassfrogs are highly susceptible to changes in habitat conditions, they mostly occur in inaccessible (and unaltered) areas of the rainforest and cloud forest (e.g., *Nymphargus garciae*, Ruiz-Carranza and Lynch 1995; *Nymphargus humboldti*, Guayasamin et al. 2020; *Teratohyla adenocheira*, Harvey and Noonan 2005). In addition, the ecology of some glassfrog species makes it difficult to record their calls because they are explosive breeders. This means that they are available only for a limited time and under specific environmental conditions, and the males perch and call near waterfalls where they usually go unnoticed due to the high level of background noise (e.g., "*Centrolene*" *medemi, C. gekoidea,* and *R. adiazeta*; Ruíz-Carranza and Lynch 1991; Lynch et al. 1983). Finally, low population densities or population decreases could reduce the detectability of these species in the field (e.g., "*Centrolene*" *acantidiocephalum*, Ruiz-Carranza and Lynch 1989; "*Centrolene*" *azulae*, Flores and McDiarmid 1989), making recordings of their calls rare or non-existent.

The new call descriptions for four glassfrog species presented here, plus the call descriptions of two species in strategic localities, contribute to the knowledge of endemic amphibian species that occur in the northern Andes. In addition, our results highlight the glassfrog species that do not have their call described thus far, and which should be prioritized in bioacoustic studies due to their threat status, endemism, and lack of occurrence in protected areas. This information can be used to establish future programs that use acoustic recordings to monitor populations with automated records, and to explore the taxonomic, ecological, and behavioral aspects of other understudied species.

Acknowledgments.—AMMH was supported by scholarship 416922 from Consejo Nacional de Ciencia y Tecnología (CONACyT, Mexico), through Posgrado de Ciencias Biológicas of the Universidad Nacional Autónoma de México (UNAM). This project was supported by PAPIIT-DGAPA (UNAM) project 203617 and the Rufford Foundation (Rufford Small Grant reference 18423-1). Funding for MR was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES code 001 (PEC-PG) and PNPD programs (Proc. 2016.1.263.41.6). We sincerely thank all the people who supported us during field trips: Alejandro Rodriguez, Jose Criollo, Arnol C. Sanchez, Valentina Nieto, José Vieira, Michael Pasaje, Maria Camila Basto-Riascos, and Davixon Jiménez Ferreira. Finally, we thank Johana Goyes and Taggert Butterfield, and two anonymous reviewers, for their invaluable comments on the manuscript.

Literature Cited

Almendáriz A, Batallas D. 2012. Nuevos datos sobre la distribución, historia natural y el canto de *Centrolene condor* Cisneros-Heredia y Morales-Mite, 2008 (Amphibia: Anura: Centrolenidae). *Revista Politécnica* 30(3): 42–53.

Barrett K, Nibbelink NP, Maerz JC. 2014. Identifying priority species and conservation opportunities under future climate scenarios: amphibians in a biodiversity hotspot. *Journal of Fish and Wildlife Management* 5: 282–297.

Bravo-Valencia L, Delia JRJ. 2016. Maternal care in a glassfrog: care function and commitment to offspring in *Ikakogi tayrona*. *Behavioral Ecology and Sociobiology* 70: 41–48.

Brooks TM, Bakarr MI, Boucher T, Da Fonseca GA, Hilton-Taylor C, Hoekstra JM, Moritz T, Olivieri S, Parrish J, Pressey RL, et al. 2004. Coverage provided by the global protected-area system: is it enough? *BioScience* 54: 1,081–1,091.

Cadle JE, McDiarmid RW. 1990. Two new species of *Centrolenella* (Anura: Centrolenidae) from northwestern Peru. *Proceedings of the Biological Society of Washington* 103: 746–768.

Caminer MA, Mila B, Jansen M, Fouquet A, Venegas PJ, Chavez G, Lougheed SC, Ron SR. 2017. Systematics of the *Dendropsophus leucophyllatus* species complex (Anura: Hylidae): cryptic diversity and the description of two new species. *PLoS One* 12: e0171785.

Cardozo-Urdaneta A, Señaris JC. 2012. Vocalización y biología reproductiva de las ranas de cristal *Hyalinobatrachium pallidum* y *Centrolene daidaleum* (Anura, Centrolenidae) en la sierra de Perijá, Venezuela. *Memoria de la Fundación La Salle de Ciencias Naturales* 70(173–174): 87–105.

Carvajal-Endara S, Coloma LA, Morales-Mite MA, Guayasamin JM, Szekely P, Duellman WE. 2019. Phylogenetic systematics, ecology, and conservation of marsupial frogs (Anura: Hemiphractidae) from the Andes of southern Ecuador, with descriptions of four new biphasic species. *Zootaxa* 4562: 1–102.

Catenazzi A, Rodríguez LO, Donnelly MA. 2009. The

advertisement calls of four species of glassfrogs (Centrolenidae) from southeastern Peru. *Studies on Neotropical Fauna and Environment* 44: 83–91.

Chavez G, Pradel R, Catenazzi A. 2019. Integrative taxonomy reveals first country record of *Hyalinobatrachium mondolfii* Señaris and Ayarzagüena, 2001, and distribution range extensions for *Cochranella nola* Harvey, 1996, and *Rulyrana spiculata* Duellman, 1976 (Anura: Centrolenidae) in Peru. *Zootaxa* 4691: 541–560.

Connelly S, Pringle CM, Whiles MR, Lips KR, Kilham S, Brenes R. 2011. Do tadpoles affect leaf decomposition in neotropical streams? *Freshwater Biology* 56: 1,863–1,875.

Dautel N, Maldonado ALS, Abuza R, Imba H, Griffin K, Guayasamin JM. 2011. Advertisement and combat calls of the glassfrog *Centrolene lynchi* (Anura: Centrolenidae), with notes on combat and reproductive behaviors. *Phyllomedusa: Journal of Herpetology* 10: 31–43.

Delia J, Ramirez-Bautista A, Summers K. 2014. Glassfrog embryos hatch early after parental desertion. *Proceedings of the Royal Society B: Biological Sciences* 281(1785): 1–7.

Delia J, Bravo-Valencia L, Warkentin KM. 2017. Patterns of parental care in neotropical glassfrogs: fieldwork alters hypotheses of sex-role evolution. *Journal of Evolutionary Biology* 30(5): 898–914.

Flores G, McDiarmid RW. 1989. Two new species of South American *Centrolenella* (Anura: Centrolenidae) related to *C. mariae*. *Herpetologica* 45: 401–411.

Frost DR. 2021. Amphibian Species of the World: an Online Reference. Version 6.1. American Museum of Natural History, New York, New York, USA. Available: https://amphibiansoftheworld.amnh.org/index.php [Accessed: 15 September 2020].

Galindo CA, Vichue-Lozano A, Bernal MH. 2020. The advertisement call of the Colombian endemic glassfrog *Rulyrana susatamai* (Anura: Centrolenidae). *Zootaxa* 4852: 586–589.

Grant T, Bolivar GW, Castro F. 1998. The advertisement call of *Centrolene geckoideum*. *Journal of Herpetology* 32: 452–455.

Guayasamin JM, Castroviejo-Fisher S, Trueb L, Ayarzagüena J, Rada M, Vilà C. 2009. Phylogenetic systematics of glassfrogs (Amphibia: Centrolenidae) and their sister taxon *Allophryne ruthveni*. *Zootaxa* 2100: 1–97.

Guayasamin JM, Cisneros-Heredia DF, Vieira J, Kohn S, Gavilanes G, Lynch RL, Hamilton PS, Maynard RJ. 2019. A new glassfrog (Centrolenidae) from the Chocó-Andean Río Manduriacu Reserve, Ecuador, endangered by mining. *PeerJ* 7: e6400.

Guayasamin JM, Cisneros-Heredia DF, McDiarmid RW, Peña P, Hutter CR. 2020. Glassfrogs of Ecuador: diversity, evolution, and conservation. *Diversity* 12: 222.

Guerra V, Llusia D, Gambale PG, Morais ARD, Marquez R, Bastos RP. 2018. The advertisement calls of Brazilian anurans: historical review, current knowledge, and future directions. *PLoS One* 13: e0191691.

Harvey MB, Noonan BP. 2005. Bolivian glassfrogs (Anura: Centrolenidae) with a description of a new species from Amazonia. *Proceedings of the Biological Society of Washington* 118: 428–441.

Hutter CR, Guayasamin JM. 2012. A new cryptic species of glassfrog (Centrolenidae: *Nymphargus*) from Reserva Las Gralarias, Ecuador. *Zootaxa* 3257: 1–21.

Hutter CR, Guayasamin JM, Wiens JJ. 2013. Explaining Andean megadiversity: the evolutionary and ecological causes of glassfrog elevational richness patterns. *Ecology Letters* 16: 1,135–1,144.

IUCN. 2019. The IUCN Red List of Threatened Species. Version 2019-1. Available: https://www.iucnredlist.org [Accessed: 15 September 2020].

Köhler J, Jansen M, Rodriguez A, Kok PJ, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods, and recommendations for best practice. *Zootaxa* 4251: 1–124.

Kubicki B. 2007. *Ranas de Vidrio de Costa Rica/Glass Frogs of Costa Rica*. Editorial INBio, Santo Domingo de Heredia, Costa Rica. 304 p.

Laiolo P. 2010. The emerging significance of bioacoustics in animal species conservation. *Biological Conservation* 143: 1,635–1,645.

Lips KR, Burrowes PA, Mendelson III JR, Parra-Olea G. 2005. Amphibian declines in Latin America: widespread population declines, extinctions, and impacts. *Biotropica* 37: 163–165.

Loyola RD, Nabout JC, Trindade Filho J, Lemes P, Cardona JNU, Dobrovolski R, Sagnori MD, Diniz Filho JAF. 2012. Climate change might drive species into reserves: a case study of the American Bullfrog in the Atlantic Forest Biodiversity Hotspot. *Alytes* 29: 61–74.

Lynch JD, Ruiz-Carranza PM, Rueda-Almonacid JV. 1983. Notes on the distribution and reproductive biology of *Centrolene geckoideum* Jimenez de la Espada in Colombia and Ecuador (Amphibia: Centrolenidae). *Studies on Neotropical Fauna and Environment* 18: 239–243.

Márquez R, De la Riva I, Bosch J. 1996. Advertisement calls of three glassfrogs from the Andean forests (Amphibia: Anura: Centrolenidae). *Herpetological Journal* 6: 97–99.

Mendoza AM, Arita HT. 2014. Priority setting by sites and by species using rarity, richness, and phylogenetic diversity: the case of neotropical glassfrogs (Anura: Centrolenidae). *Biodiversity and Conservation* 23: 909–926.

Mendoza-Henao AM, Basto-Riascos MC, Rada M. 2019. *Centrolene huilensis* (Ruiz-Carraza and Lynch, 1995). *Catálogo de Anfibios y Reptiles de Colombia* 5: 18–23.

Padial JM, De la Riva I. 2009. Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). *Zoological Journal of the Linnean Society* 155: 97–122.

Peterson CR, Dorcas ME. 1994. Automated data acquisition. Pp. 47–57 In: *Measuring and Monitoring Biological Diversity*. Editors, Heyer RW, Donnelly MA, McDiarmid RW, Hayek LAC, and Foster SM. Smithsonian Tropical Institute, Washington, DC, USA. 364 p.

Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MP, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439: 161–167.

Ríos-Soto J, Ospina-L AM, Vargas-Salinas F. 2017. The advertisement call and notes of the reproductive ecology of the glassfrog *"Centrolene" quindianum* (Anura: Centrolenidae). *South American Journal of Herpetology* 12(2): 117–127.

Rivera-Correa M, Ospina-L AM, Rojas-Montoya M, Venegas-Valencia K, Rueda-Solano LA, Gutiérrez-Cárdenas PDA, Vargas-Salinas F. 2021 Cantos en ranas y sapos de Colombia: estado del conocimiento y perspectivas de investigación en ecoacústica. *Neotropical Biodiversity* 7: 350–363.

Rodrigues AS, Akcakaya HR, Andelman SJ, Bakarr MI, Boitani L, Brooks TM, Chanson JS, Fishpool LDC, Da Fonseca GAB, Gaston KJ, et al. 2004. Global gap analysis: priority regions for expanding the global protected-area network. *BioScience* 54: 1,092–1,100.

Rodriguez A, Dugo-Cota A, Montero-Mendieta S, Gonzalez-Voyer A, Alonso R, Vences M, Vila C. 2017. Cryptic within cryptic: genetics, morphometrics, and bioacoustics delimitate a new species of *Eleutherodactylus* (Anura: Eleutherodactylidae) from Eastern Cuba. *Zootaxa* 4221: 501–522.

Ruiz-Carranza PM, Lynch JD. 1989. Una nueva especie de *Centrolenella* Noble, 1920 (Amphibia: Anura: Centrolenidae) de la Cordillera Oriental de Colombia. *Trianea* 3: 67–75.

Ruiz-Carranza PM, Lynch JD. 1991a. Ranas Centrolenidae de Colombia I. Propuesta de una nueva clasificacion generica. *Lozania* 57: 1–30.

Ruiz-Carranza PM, Lynch JD. 1991b. Ranas Centrolenidae de Colombia II. Nuevas especies de *Centrolene* de la Cordillera Oriental y Sierra Nevada de Santa Marta. *Lozania* 58: 1–26.

Ruiz-Carranza PM, Lynch JD. 1991c. Ranas Centrolenidae de Colombia IV. Nuevas especies de *Cochranella* del grupo *ocellata* de la Cordillera Oriental. *Lozania* 60: 1–13.

Ruiz-Carranza PM, Lynch JD. 1995. Ranas Centrolenidae de Colombia. V. Cuatro nuevas especies de *Cochranella* de la Cordillera Central. *Lozania* 62: 1–23.

Ruiz-Carranza PM, Lynch JD. 1995b. Ranas Centrolenidae de Colombia VIII. Cuatro nuevas especies de Centrolene de la Cordillera Central. *Lozania* 65: 1–16.

Salgado AL, Guayasamin JM. 2018. Parental care and reproductive behavior of the Minute Dappled Glassfrog (Centrolenidae: *Centrolene peristictum*). *South American Journal of Herpetology* 13(3): 211–219.

Sánchez-Giraldo C, Bedoya CL, Morán-Vásquez RA, Isaza CV, Daza JM. 2020. Ecoacoustics in the rain: understanding acoustic indices under the most common geophonic source in tropical rainforests. *Remote Sensing in Ecology and Conservation* 6: 248–261.

Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, Catenazzi A, et al. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363: 1,459–1,463.

Señaris JC, Ayarzagüena J. 2005. Revisión taxonómica de la familia Centrolenidae (Amphibia; Anura) de Venezuela. *Publicaciones del Comité Español del Programa Hombre y Biosfera - Red IberoMaB de la UNESCO* 7: 1–337.

Stuart SN, Hoffmann M, Chanson J, Cox N, Berridge R, Ramani P, Young B. 2008. *Threatened Amphibians of the World*. Lynx Editions, Barcelona Spain; IUCN, Gland, Switzerland; and Conservation International, Arlington, Virginia, USA. 758 p.

Twomey E, Delia J, Castroviejo-Fisher S. 2014. A review of northern Peruvian glassfrogs (Centrolenidae), with the description of four new remarkable species. *Zootaxa* 3851: 1–87.

Valencia-Aguilar A, Cortés-Gómez AM, Ruiz-Agudelo CA. 2013. Ecosystem services provided by amphibians and reptiles in Neotropical ecosystems. *International Journal of Biodiversity Science, Ecosystem Services, and Management* 93: 257–272.

Vargas-Salinas F, Ospina-L AM, Rios-Soto JA, Rivera-Correa M. 2017. *Centrolene savagei. Catalogue of American Amphibians and Reptiles* 912: 1–12.

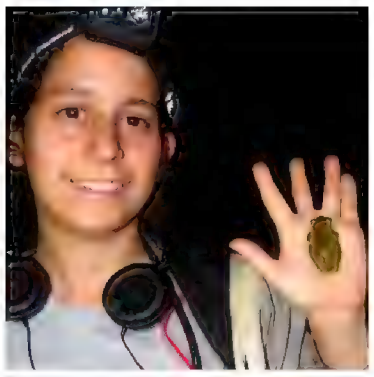
Viuche-Lozano A, Enciso-Calle MP, Bernal MH. 2018. The advertisement call of *Centrolene notostictum* (Anura, Centrolenidae) with a new record of geographic distribution in Tolima, Colombia. *Zootaxa* 4377: 575–576.

Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105(Supplement 1): 11,466–11,473.

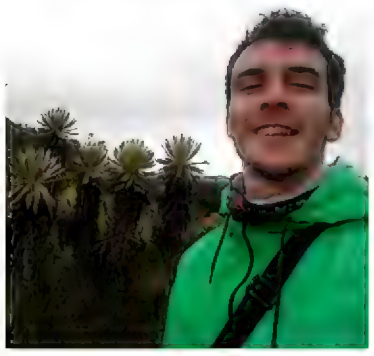
Whiles MR, Hall RO Jr, Dodds WK, Verburg P, Huryn AD, Pringle CM, Lips KR, Kilham SS, Colón-Gaud C, Rugenski AT, et al. 2013. Disease-driven amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems* 16: 146–157.

Whiles MR, Lips KR, Pringle CM, Kilham SS, Bixby RJ, Brenes R, Conelly S, Colon-Gaud JC, Hunte-Brown M, Huryn AD. 2006. The effects of amphibian population decline on the structure and function of Neotropical stream ecosystems. *Frontiers in Ecology*

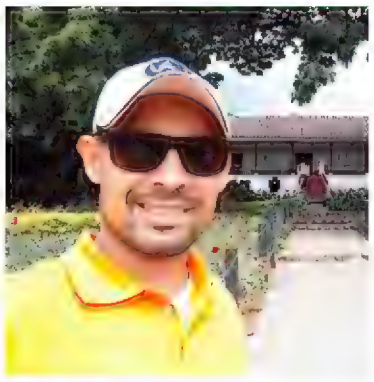
and the Environment 4: 27–34.
Zipkin EF, DiRenzo GV, Ray JM, Rossman S, Lips KR. 2020. Tropical snake diversity collapses after widespread amphibian loss. *Science* 367: 814–816.



Angela M. Mendoza-Henao is a biologist from Universidad del Valle (Cali, Colombia) with an M.Sc. and Ph.D. in Biological Sciences from the Universidad Nacional Autónoma de México (UNAM). Angela's work has focused mainly in the application of molecular tools and bioacoustics for answering questions in ecology and conservation, with an emphasis on terrestrial vertebrates, mainly neotropical amphibians.



Sebastián Duarte-Marín is a biologist from Universidad del Quindío (Armenia, Colombia) and co-founder of the biodiversity illustration company Entropia. Sebastián's work has focused mainly on the study of ecology, taxonomy, and bioacoustics of neotropical amphibians, particularly on frogs with direct development and glassfrogs.



Marco Rada is a biologist from Pontificia Universidad Javeriana, Bogotá (PUJ-Colombia) with a Ph.D. from the Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (PUCRS-Brazil). Marcos's work has focused mainly in the use of interdisciplinary tools for answering questions in taxonomy and phylogenetic relationships, with an emphasis on neotropical amphibians, including glassfrogs.

Appendix 1. Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
Celsiella revocata	Yes	Vulnerable (VU)	Subregional	Yes	Señaris and Ayarzagüena 2005
Celsiella vozmedianoi	No	Data Deficient (DD)	Local	Yes	
"Centrolene" acanthidiocephalum	No	Data Deficient (DD)	Local	No	Gonzalez-Durán et al., in prep.
Centrolene altitudinalis	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005
Centrolene antioquiensis	No	Near Threatened (NT)	Subregional	No	Duarte-Marín et al., in prep.
"Centrolene" azulae	No	Data Deficient (DD)	Local	No	
Centrolene ballux	Yes	Endangered (EN)	Local	No	Márquez et al. 1996
Centrolene buckleyi	Yes	Vulnerable (VU)	Regional	No	Guayasamin et al. 2006
Centrolene charapita	No	Critically Endangered (CR)	Local	No	
Centrolene condor	Yes	Data Deficient (DD)	Local	No	Almenzariz-C and Batalla 2012
Centrolene daidalea	Yes	Vulnerable (VU)	Subregional	No	Cardozo-Urdaneta and Señaris 2012
Centrolene geckoidea	Yes	Critically Endangered (CR)	Subregional	No	Grant et al. 1998
Centrolene heloderma	Yes	Vulnerable (VU)	Subregional	Yes	Guayasamin et al. 2020

Appendix 1 (continued). Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
Centrolene hesperia	Yes	Endangered (EN)	Local	Yes	Cadle and McDiarmid 1990
Centrolene huilensis	Yes	Endangered (EN)	Local	No	This study
Centrolene hybrida	Yes	Least Concern (LC)	Subregional	Yes	This study
Centrolene lemniscata	No	Data Deficient (DD)	Local	Yes	
Centrolene lynchi	Yes	Endangered (EN)	Subregional	No	Dautel et al. 2011
"Centrolene" medemi	No	Endangered (EN)	Subregional	No	
Centrolene muelleri	No	Data Deficient (DD)	Local	Yes	
Centrolene notosticta	Yes	Least Concern (LC)	Subregional	No	Viuche-Lozano et al. 2018
Centrolene paezorum	No	Data Deficient (DD)	Local	No	
Centrolene peristicta	Yes	Least Concern (LC)	Regional	No	Salgado and Guayasamin 2018
"Centrolene" petrophilum	No	Endangered (EN)	Local	No	
Centrolene pipilatum	No	Endangered (EN)	Local	Yes	
"Centrolene" quindianum	Yes	Vulnerable (VU)	Subregional	No	Ríos-Soto et al. 2017
"Centrolene" robledoi	No	Least Concern (LC)	Regional	Yes	Duarte-Marín et al., in prep.
Centrolene sabini	Yes	Vulnerable (VU)	Local	Yes	Catenazzi et al. 2009, 2012
Centrolene sanchezi	Yes	Data Deficient (DD)	Local	Yes	Guayasamin et al. 2020
Centrolene savagei	Yes	Least Concern (LC)	Subregional	Yes	Díaz-Gutiérrez et al. 2013
Centrolene solitaria	No	Endangered (EN)	Local	No	Basto-Riascos et al., in prep.
Centrolene venezuelense	Yes	Least Concern (LC)	Subregional	Yes	Señaris and Ayarzagüena 2005
Chimerella corleone	Yes	Data Deficient (DD)	Local	No	Twomey et al. 2014
Chimerella mariaelenae	Yes	Least Concern (LC)	Regional	No	Batallas and Brito 2016
"Cochranella" duidaeana	No	Data Deficient (DD)	Local	Yes	
Cochranella erminea	Yes	Least Concern (LC)	Regional	No	Twomey et al. 2014
"Cochranella" euhystrix	No	Critically Endangered (CR)	Local	No	
Cochranella euknemos	Yes	Least Concern (LC)	Regional	Yes	Savage and Starrett 1967; Kubicki 2007
"Cochranella" geijskesi	No	Least Concern (LC)	Local	Yes	
Cochranella granulosa	Yes	Least Concern (LC)	Regional	No	Ibáñez 1993; Kubicki 2007
Cochranella guayasamini	Yes	Least Concern (LC)	Subregional	No	Twomey et al. 2014
Cochranella litoralis	No	Vulnerable (VU)	Subregional	Yes	
Cochranella mache	Yes	Endangered (EN)	Local	Yes	Ortega-Andrade et al. 2013
"Cochranella" megistra	No	Endangered (EN)	Subregional	No	
Cochranella nola	Yes	Near Threatened (NT)	Subregional	No	Lötters and Köhler 2000
"Cochranella" ramirezi	No	Data Deficient (DD)	Local	No	
Cochranella resplendens	No	Least Concern (LC)	Global	Yes	

Appendix 1 (continued). Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
"Cochranella" riveroi	No	Vulnerable (VU)	Local	Yes	
"Cochranella" xanthocheridia	No	Vulnerable (VU)	Subregional	No	
Espadarana andina	Yes	Least Concern (LC)	Subregional	No	Señaris and Ayarzagüena 2005; Cabanzo-Olarte and Ortega-Chinchilla 2017
Espadarana audax	Yes	Least Concern (LC)	Regional	Yes	Duarte-Marín et al., in prep.; Guayasamin et al. 2020
Espadarana callistomma	Yes	Least Concern (LC)	Regional	Yes	Guayasamin et al. 2020
Espadarana durrellorum	No	Least Concern (LC)	Regional	No	
Espadarana prosoblepon	Yes	Least Concern (LC)	Global	Yes	Jacobson 1985; Kubicki 2007
Hyalinobatrachium adespinosai	Yes	Data Deficient (DD)	Local	No	Guayasamin et al. 2019
Hyalinobatrachium anachoretus	Yes	Endangered (EN)	Local	No	Twomey et al. 2014
Hyalinobatrachium aureoguttatum	No	Near Threatened (NT)	Subregional	No	
Hyalinobatrachium bergeri	Yes	Least Concern (LC)	Regional	Yes	Castroviejo-Fisher et al. 2009
Hyalinobatrachium cappellei	Yes	Not evaluated	Regional	Yes	Myers and Donelly 1997, 2001; Señaris and Ayarzagüenza 2005; Castroviejo-Fisher et al. 2011; Oliveira et al. 2015
Hyalinobatrachium carlesvilai	Yes	Not evaluated	Regional	No	Márquez et al. 1996; Castroviejo-Fisher et al. 2009
Hyalinobatrachium chirripoi	Yes	Least Concern (LC)	Subregional	Yes	Kubicki 2007; Kubicki et al. 2015
Hyalinobatrachium colymbiphyllum	Yes	Least Concern (LC)	Regional	No	Kubicki 2007; Kubicki et al. 2015
Hyalinobatrachium dianae	Yes	Data Deficient (DD)	Local	Yes	Kubicki et al. 2015
Hyalinobatrachium duranti	Yes	Data Deficient (DD)	Subregional	Yes	Señaris and Ayarzagüena 2005
Hyalinobatrachium esmeralda	Yes	Endangered (EN)	Local	No	Acosta-Galvis 2017
Hyalinobatrachium fleischmanni	Yes	Least Concern (LC)	Global	Yes	Jacobson 1985; Kubicki 2007; Castroviejo-Fisher et al. 2011; Greer and Wells 1980
Hyalinobatrachium fragile	Yes	Vulnerable (VU)	Subregional	Yes	Wen et al. 2012
Hyalinobatrachium guairarepanense	Yes	Endangered (EN)	Local	Yes	Señaris and Ayarzagüena 2005
Hyalinobatrachium iaspidiense	Yes	Data Deficient (DD)	Subregional	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2011
Hyalinobatrachium ibama	No	Vulnerable (VU)	Subregional	No	
Hyalinobatrachium kawense	Yes	Least Concern (LC)	Regional	No	Castroviejo-Fisher et al. 2011
Hyalinobatrachium mesai	Yes	Not evaluated	Local	Yes	Castroviejo-Fisher et al. 2011
Hyalinobatrachium mondolfii	Yes	Least Concern (LC)	Regional	No	Señaris and Ayarzagüena 2001; Castroviejo-Fisher et al. 2011

Appendix 1 (continued). Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
Hyalinobatrachium muiraquitan	No	Not evaluated	Local	No	
Hyalinobatrachium munozorum	Yes	Least Concern (LC)	Global	Yes	Guayasamin et al. 2020
Hyalinobatrachium orientale	Yes	Vulnerable (VU)	Subregional	No	Castroviejo-Fisher et al. 2008; Cardozo-Urdaneta and Señaris 2012
Hyalinobatrachium orocostale	Yes	Not evaluated	Subregional	Yes	Castroviejo-Fisher et al. 2008
Hyalinobatrachium pallidum	Yes	Endangered (EN)	Local	Yes	Cardozo-Urdaneta and Señaris 2012
Hyalinobatrachium pellucidum	Yes	Near Threatened (NT)	Local	Yes	Wen et al. 2012
Hyalinobatrachium talamancae	Yes	Least Concern (LC)	Subregional	Yes	Kubicki 2007; Kubicki et al. 2015
Hyalinobatrachium tatayoi	Yes	Least Concern (LC)	Local	Yes	Castroviejo-Fisher et al. 2007
Hyalinobatrachium taylori	Yes	Least Concern (LC)	Global	Yes	Señaris and Ayarzagüena 2005
Hyalinobatrachium tricolor	Yes	Least Concern (LC)	Regional	Yes	Castroviejo-Fisher et al. 2011
Hyalinobatrachium valerioi	Yes	Least Concern (LC)	Regional	No	Kubicki 2007; Kubicki et al. 2015
Hyalinobatrachium vireovittatum	Yes	Data Deficient (DD)	Local	No	Kubicki 2007; Kubicki et al. 2015
Hyalinobatrachium yaku	Yes	Data Deficient (DD)	Local	No	Guayasamin et al. 2017
Ikakogi ispacue	Yes	Not evaluated	Local	Yes	Rada et al. 2019
Ikakogi tayrona	Yes	Vulnerable (VU)	Subregional	Yes	Vargas-Salinas et al. 2015
Nymphargus anomalus	No	Critically Endangered (CR)	Local	Yes	
Nymphargus armatus	No	Critically Endangered (CR)	Local	No	
Nymphargus balionotus	No	Vulnerable (VU)	Subregional	No	
Nymphargus bejaranoi	Yes	Least Concern (LC)	Regional	No	Márquez et al. 1996
Nymphargus buenaventura	No	Data Deficient (DD)	Local	No	
Nymphargus caritiocommatus	No	Data Deficient (DD)	Local	Yes	
Nymphargus caucanus	No	Endangered (EN)	Local	Yes	Duarte-Marín et al., in prep.
Nymphargus chami	No	Data Deficient (DD)	Subregional	No	Duarte-Marín et al., in prep.
Nymphargus chancas	No	Data Deficient (DD)	Local	Yes	
Nymphargus cochranae	No	Vulnerable (VU)	Subregional	Yes	
Nymphargus colomai	No	Vulnerable (VU)	Local	No	
Nymphargus cristinae	No	Data Deficient (DD)	Local	No	
Nymphargus garciae	No	Vulnerable (VU)	Subregional	Yes	González-Acosta et al., in prep.
Nymphargus grandisonae	Yes	Least Concern (LC)	Subregional	No	Hutter et al. 2013
Nymphargus griffithsi	Yes	Least Concern (LC)	Regional	No	Hutter and Guayasamin 2012; Arcila-Perez et al. 2017
Nymphargus humboldti	No	Data Deficient (DD)	Subregional	Yes	
Nymphargus ignotus	No	Least Concern (LC)	Subregional	No	Duarte-Marín et al., in prep.

Appendix 1 (continued). Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
Nymphargus lasgralarias	Yes	Endangered (EN)	Local	Yes	Hutter and Guayasamin 2012
Nymphargus laurae	No	Critically Endangered (CR)	Local	No	
Nymphargus lindae	No	Endangered (EN)	Local	No	
Nymphargus luminosus	No	Endangered (EN)	Local	No	
Nymphargus luteopunctatus	No	Endangered (EN)	Local	No	
Nymphargus manduriacu	Yes	Critically Endangered (CR)	Local	Yes	Guayasamin et al. 2019
Nymphargus mariae	Yes	Least Concern (LC)	Regional	No	Guayasamin et al. 2020
Nymphargus megacheirus	No	Endangered (EN)	Local	Yes	
Nymphargus mixomaculatus	No	Critically Endangered (CR)	Local	Yes	
Nymphargus nephelophila	No	Data Deficient (DD)	Local	No	
Nymphargus ocellatus	No	Data Deficient (DD)	Local	No	
Nymphargus oreonympha	Yes	Data Deficient (DD)	Local	No	This study
Nymphargus phenax	No	Endangered (EN)	Local	No	
Nymphargus pluvialis	Yes	Data Deficient (DD)	Local	No	Catenazzi et al. 2009
Nymphargus posadae	No	Least Concern (LC)	Regional	No	
Nymphargus prasinus	No	Vulnerable (VU)	Subregional	No	
Nymphargus rosada	No	Vulnerable (VU)	Subregional	No	Duarte-Marín et al., in prep.
Nymphargus ruizi	No	Vulnerable (VU)	Subregional	Yes	
Nymphargus siren	Yes	Vulnerable (VU)	Subregional	Yes	Guayasamin et al. 2020
Nymphargus spilotus	No	Near Threatened (NT)	Local	Yes	Duarte-Marín et al., in prep.
Nymphargus sucre	No	Critically Endangered (CR)	Local	No	
Nymphargus truebae	Yes	Critically Endangered (CR)	Local	No	Catenazzi et al. 2009
Nymphargus vicenteruedai	No	Data Deficient (DD)	Local	Yes	
Nymphargus wileyi	No	Data Deficient (DD)	Local	Yes	
Rulyrana adiazeta	No	Vulnerable (VU)	Subregional	No	
Rulyrana flavopunctata	Yes	Least Concern (LC)	Regional	No	Guayasamin et al. 2020
Rulyrana mcdiarmidi	No	Data Deficient (DD)	Subregional	No	
Rulyrana saxiscandens	Yes	Endangered (EN)	Local	No	Twomey et al. 2014
Rulyrana spiculata	Yes	Near Threatened (NT)	Subregional	No	Catenazzi et al. 2009
Rulyrana susatamai	Yes	Near Threatened (NT)	Subregional	No	Galindo et al. 2020; This study
Sachatamia albomaculata	Yes	Least Concern (LC)	Regional	No	Kubicki 2007
Sachatamia electrops	No	Endangered (EN)	Local	No	
Sachatamia ilex	Yes	Least Concern (LC)	Regional	No	Kubicki 2007; Guayasamin et al. 2020
Sachatamia orejuela	No	Least Concern (LC)	Subregional	No	Duarte-Marín et al., in prep.
Sachatamia punctulata	Yes	Vulnerable (VU)	Subregional	No	This study
Teratohyla adenocheira	No	Data Deficient (DD)	Local	Yes	
Teratohyla amelie	Yes	Data Deficient (DD)	Local	No	Guayasamin et al. 2020

Appendix 1 (continued). Status of glassfrog species with respect to whether or not their advertisement call has been described, conservation status, distributional range, and occurrence in Natural Protected Areas.

Species	Call described	IUCN category	Endemism	Protected areas	Author
Teratohyla midas	Yes	Least Concern (LC)	Global	Yes	Araújo et al. 2018; Guayasamin et al. 2020
Teratohyla pulverata	Yes	Least Concern (LC)	Regional	No	Ibañez et al 1999; Savage 2002
Teratohyla spinosa	Yes	Least Concern (LC)	Regional	No	Ibañez et al 1999; Savage 2002; Kubicki 2007
Vitreorana antisthenesi	Yes	Vulnerable (VU)	Subregional	Yes	Wen et al. 2012
Vitreorana baliomma	Yes	Not evaluated	Local	Yes	Lee Bang et al. 2019
Vitreorana castroviejoi	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2009
Vitreorana eurygnatha	Yes	Least Concern (LC)	Global	No	Heyer et al. 1990; Santana et al. 2015
Vitreorana franciscana	Yes	Not evaluated	Local	Yes	Santana et al. 2015
Vitreorana gorzulae	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2009
Vitreorana helenae	Yes	Data Deficient (DD)	Local	Yes	Señaris and Ayarzagüena 2005; Castroviejo-Fisher et al. 2009
Vitreorana parvula	No	Data Deficient (DD)	Local	No	
Vitreorana ritae	Yes	Data Deficient (DD)	Subregional	Yes	Señaris and Ayarzagüena 2005; Cisneros-Heredia 2013
Vitreorana uranoscopa	Yes	Least Concern (LC)	Global	Yes	Heyer 1985; Haga et al. 2014

Appendix 2. List of highly threatened (IUCN classifications Endangered (EN) or Critically Endangered (CR)) and understudied (IUCN categories Data Deficient (DD) or Not evaluated) glassfrog species with undescribed calls and a range that either includes less than five known locations in less than 1,500 km^2 or does not overlap with any protected areas. Asterisks indicate species for which the calls are currently being described.

Highly threatened species (EN or CR)	Understudied species (DD or Not evaluated)
Centrolene charapita	*"Centrolene" acanthidiocephalum**
"Centrolene" petrophilum	*"Centrolene" azulae*
*Centrolene solitaria**	*Centrolene paezorum*
"Cochranella" euhystrix	*"Cochranella" ramirezi*
Nymphargus armatus	*Hyalinobatrachium muiraquitan*
Nymphargus laurae	*Nymphargus buenaventura*
Nymphargus lindae	*Nymphargus colomai*
Nymphargus luminosus	*Nymphargus cristinae*
Nymphargus luteopunctatus	*Nymphargus nephelophila*
Nymphargus phenax	*Nymphargus ocellatus*
Nymphargus sucre	*Vitreorana parvula*
Sachatamia electrops	